

PCT International Application No. GB97/03400, filed December 10, 1997, and further

B1 claims priority to foreign application United Kingdom 9625640.9, filed December 10,

1996

On page 3, line 12 insert:

Brief Description of the Drawings

Fig. 1. SDS-PAGE analysis of PEG-modified hA5B7 Fab'. Samples of unmodified hA5B7 Fab' (lane 1); hinge-modified Fab' (lane 2), and randomly-modified Fab' (lane 3) were prepared with non-reducing sample buffer, and 1.5 µg of each loaded onto a 4-20% gradient Tris-glycine gel. Standard protein markers (lane M) were also run, comprising myosin (200 kDa), beta-galactosidase (116.3 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.3 kDa), glutamate dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), aprotinin (6 kDa) and insulin B & A chains (3.5 & 2.5 kDa). Following electrophoresis, the gel was stained with coomassie blue.

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Fig. 3. SDS-PAGE analysis of hTNF40 Fab'-PEG conjugates. Samples of hTNF40 Fab'-PEG (25 kDa) prepared by random conjugation (lane 1), Fab'-PEG (25 kDa) prepared by hinge attachment (lane 2) and Fab'-PEG (40 kDa) prepared by hinge attachment (lane 3) were prepared with non-reducing sample buffer, and 1.5 µg of each loaded onto a 4-20% gradient Tris-glycine gel. Standard protein markers (lane M) as in Fig. 1. Following electrophoresis, the gel was stained with coomassie blue.

Fig. 4. Comparison between different PEG Fab' 's, Fab' of hTNF40.4, and hTNF40.0 in

the L929 assay.

Fig. 5. Pharmacokinetics of 125-I labelled hTNF40 Fab'-PEG in rats.

Fig. 6. Pharmacokinetics of 111-In labelled hTNF40 in rats.

Fig. 7. HPLC gel filtration of hTNF40 Fab', Fab'-PEG and Fab'(PEG)₂. DuPont Zorbox GF-250 column run at 1 ml/min in 0.2 M phosphate buffer pH 7.0.

Fig. 8. Pharmacokinetics of 125-I labelled TN3 in rats.

Fig. 9. ECE15: Pharmacokinetics of 125-I labelled TN3 in rats. Fab' PEGylated via the hinge with 25K PEG.

Fig. 10. Pharmacokinetics of 125-I labelled hTNF40 Fab'-PEG in rats.

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Fig. 11. SDS-PAGE analysis under non-reducing conditions of Fab'-PEG (5 kDa) prepared using a vinylsulphone or iodoacetamide reagent. Molecular weight marker proteins (lane 1), purified Fab' (also containing F(ab')2) (lane 2), Fab'-PEG (5 kDa, VS linker) reaction mix (lane 3), Fab'-PEG (5 kDa, IA linker) reaction mix (lane 4), Fab'-PEG (5 kDa, VS linker) (lane 5) and Fab'-PEG (5 kDa, IA linker) reaction mix (lane 6).

Fig. 12a. HPLC gel filtration analysis of anti-PDGF β R Fab'-PEG reaction mix showing a peak of Fab'-PEG at 7.7 minutes and a peak of unreacted Fab' at 10.8 minutes. Fig. 12b. HPLC gel filtration analysis of purified Fab'-PEG.

Fig. 13. Pharmacokinetics of ¹²⁵I labelled anti PDGF β R IgG, Fab' and Fab'-PEG in rats.

On page 15, line 7: replace "Streamline" with — STREAMLINE—.

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